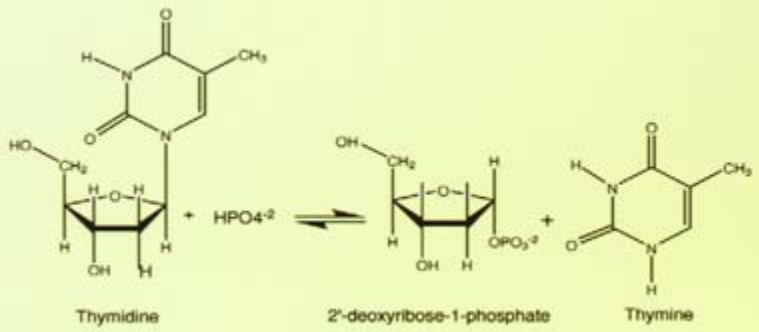
# Application of Quantum Chemistry to Biology

**A Research Project of Dr. Yuri Abashkin and Dr. Stanley K. Burt**

Quantum chemistry has not been heavily exploited for studying biological systems mainly due to limitations in computer power and memory. Computers are only now attaining speeds and memory size that allow quantum investigations of biological systems to be fruitful - but, even so, the size of the problem that can be studied is still severely limited and there is the need for new algorithmic development. Ab initio and density functional methods (DFT) offer the best hope for understanding enzyme mechanisms, hydrogen bonding, polarization effects, spectra, Van der Waals interactions and other fundamental processes in biology. We have used quantum chemistry DFT methods to study the enzyme mechanisms of HIV-1 protease, carboxypeptidase A, thymidine phosphorylase, DNA polymerase D, CDC-25, as well as to try and design mechanism-based inhibitors and anti-AIDS drugs. Given the drug resistance problem in treating HIV infection this may be an alternative, if one is clever enough. We have also used quantum chemistry DFT methods to calculate pKa's for a series dihydropyrone HIV-1 protease inhibitors for Parke-Davis and suggested substitutions that improved the compounds' potency. We have also calculated the electron affinity and reactivity for a series of compounds that cause zinc-ejection from the nucleocapsid protein P7. All of these calculations are very computationally demanding and are at the limits of the computational resources of the ABCC.

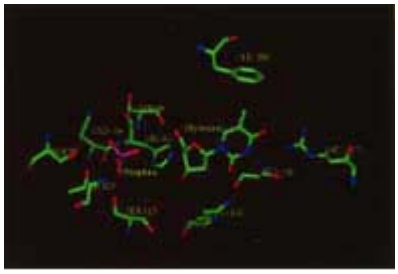
### Theoretical Investigation of the Catalytic Mechanism of Thymidine Phosphorylase

Thymidine Phosphorylase catalyzes the reversible phosphorolysis reaction of thymidine and other pyrimidine 2'-deoxyribosides as shown below.




It has been proposed that inhibitors of the pyrimidine phosphorylases might be useful and chemotherapeutic agents by interfering with the salvage of pyrimidine bases. Inhibitors might also enhance the efficacy of pyrimidine analogs such as 5-fluoro-2'deoxyuridine or 5-iodo-2'-deoxyuridine which are cleaved and thereby inactivated y pyrimidine nucleoside phosphorylases. It has also been recently reported that thymidine phosphorylase (dThyPase) is up-regulated in human colon cancer and that this overexpression correlates with increased tumor microvessel density. Several known substrate mimic inhibitors of thymidine phosphorylase are all very weak inhibitors of this enzyme. However, to our knowledge, there have not been any attempts to design a transition state analog inhibitor for thymidine phosphorylase. The goal of the present theoretical work is to attempt the design the inhibitors which are transition state mimics for Human Thymidine Phosphorylase.

We have used a recent X-ray crystal structure for E. Coli thymidine phosphorylase as a starting point in our investigation. Homology modeling of human thymidine phosphorylase indicates an exact match with the E. Coli enzyme for the residues contained in the active site of the enzyme. Molecular dynamics and molecular mechanics were used to obtain a model for the active site with the docked substrates (Figure F-1.). Density functional quantum chemical calculations, an ab initio method that allows the modeling of more atoms than traditional ab initio methods, was used to explore various mechanistic pathways for the catalyzed reaction and to locate possible transition states. In the published crystal structure of the enzyme the phosphate group is situated for away from the glycosidic bond that it attacks, and is firmly anchored to a group of charged residues and other potential hydrogen bonding residues. Thus, making an indirect attack appears to be one possible mechanism. However, it has been proposed that the catalytic reaction proceeds by a direct attack on the C1 carbon of the glycosidic bond. Therefore, we considered both a direct and an indirect reaction scheme. In all cases we found that the catalytic reaction requires protonation of thymine in the 2 position prior to cleavage of the glycosidic bond.

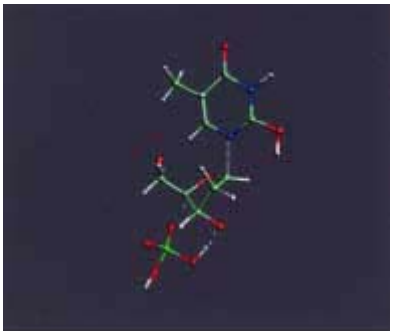


**F-1. Active site of Thymidine Phosphorylase**

**F-2a. Transition state for Direct Attack**

Two possible transition states were located. The first corresponds to a direct nucleophilic attack on the glycosidic bond (SN1) and the second to an indirect attach at 3'-OH of the deoxyribosyl ring (SN2). In the vase of a direct nucleophilic attack (Figure F-2a.), the phosphate group weakens the glycosidic bond forming an oxycarbocation at the C1' position which then reacts with the negatively charged phosphate. This transition state directly leads to the products and the corresponding reaction pathway seems intuitively reasonable from the chemical point of view. However, docking this transition state into the active site of dThyPase in the available crystal structure shows that the phosphate is located quite far from the serine residues that form the putative phosphate binding site. Thus, the significant displacement of the phosphate group from the binding site may complicate the performance of the reaction.

In the case of the indirect attack (Figure F-2b.) the phosphate attacks the 3'-OH of the ribosyl ring removing a proton and breaks the glycosidic bond. Additional rotation of the ribosyl ring is required after the cleavage of the glycosidic bond to put the C1' of the carbocation and the phosphate group close enough to obtain the products. In this reaction pathway the phosphate remains closer to its binding site in the enzyme structure. The characterization of the complete reaction profile for this system is limited to about 60 heavy atoms and required 1,000's of hours of the Cray Y-MP and J-90's.



**F-2b. Transition state for Indirect Attack**

In our restricted model both transition state structures are characterized by practically the same values of absolute energy. Thus, further experimental and theoretical work is required to verify the proposed mechanistic pathway. One factor which complicates our work is that the enzyme may be in a different conformation than found in this particular crystal structure. One structural possibility is that there is a rigid domain movement which would close the enzyme. If that is the case, then the phosphate would be placed closer to the glycosidic bond and a direct attack would be more feasible. We are trying to model this conformational change in the enzyme by molecular dynamics. Still, it should be possible from our modeling and calculations to examine databases for lead compounds corresponding to either a SN1 or SN2 reaction transition state.

### Benefits of Scalable Increases in Compute Power

The computational investigation of this limited model of Thymidine Phosphorylase required approximately 1500 CPU hours on the Cray-YMP to complete. To model this process with a larger, and more complete, active site model, including nearby protein residues, reactants, and some solvent interactions, over 250 heavy atoms are required with a concomitant increase in CPU time to over one million Cray-YMP hours. Inclusion of these additional environmental effects in the reaction model will allow the elucidation of individual contributions to the overall reaction profile. Such information will allow more effective design of transition state and mechanism-based inhibitors and enzyme reactions in the future. Therefore, a computational speed-up of three orders of magnitude is required to initiate selective investigations of there processes using realistic models. To make such calculations routine, computers that operate in the 10-100 teraflop range will be necessary.